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**HYDROGEOLOGIC INVESTIGATION, SAAD
TROUSDALE DRIVE SITE, NASHVILLE,
DAVIDSON COUNTY, TENNESSEE**

**STATUS REPORT
December 4, 1994**

Submitted to

**Roy F. Weston, Inc.
Technical Assistance Team
1021 Production Court
Louisville, KY 40299**

Submitted by

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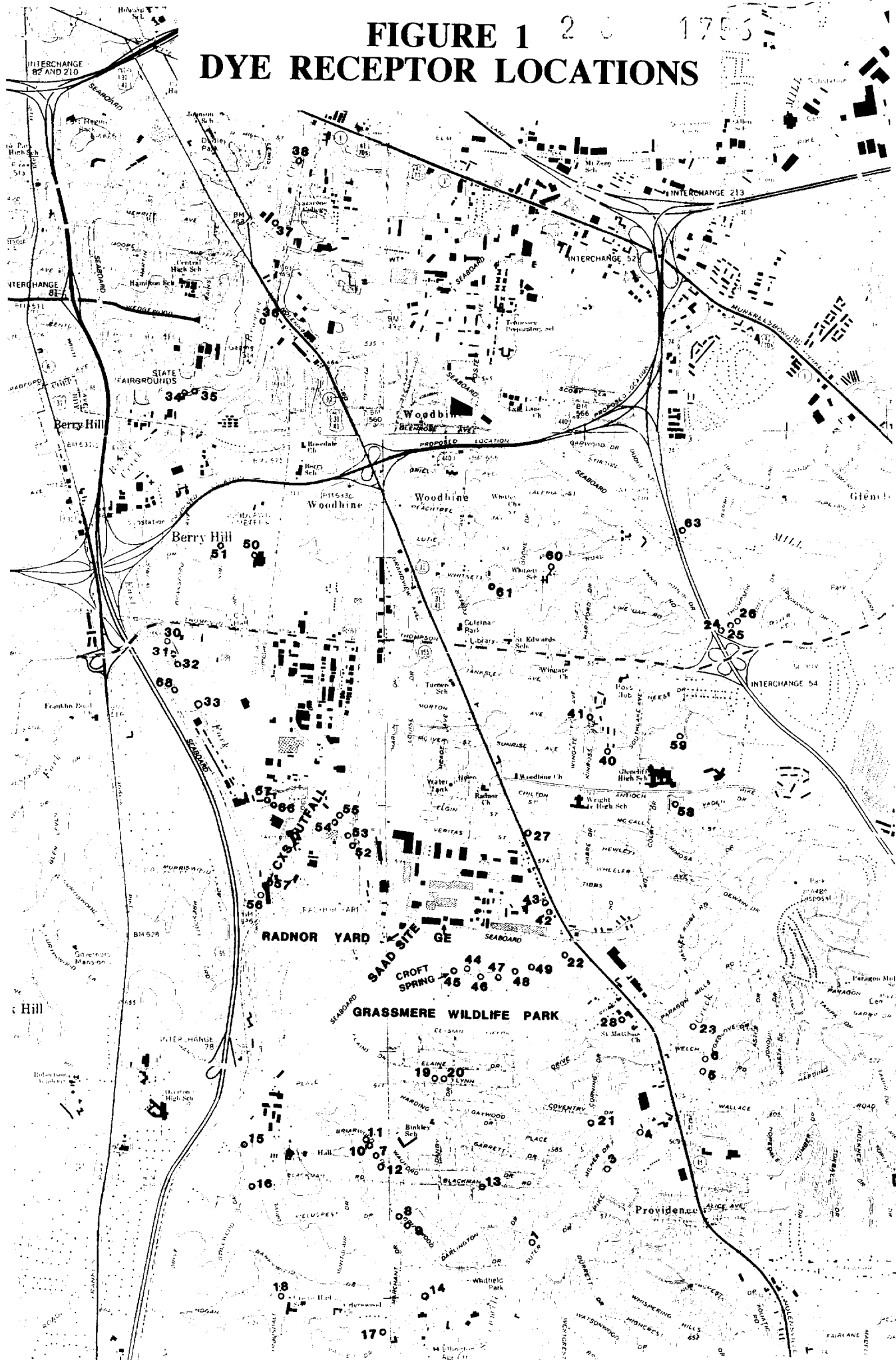
**HYDROGEOLOGIC INVESTIGATION, SAAD TROUSDALE DRIVE SITE,
NASHVILLE, DAVIDSON COUNTY, TENNESSEE**

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**HYDROGEOLOGIC INVESTIGATION, SAAD TROUSDALE DRIVE SITE,
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December 4, 1994

REASONS FOR THE SLOW MOVEMENT OF DYE THROUGH THE AQUIFER

We are not surprised by the slow movement of dyes through the aquifer. The water table is well above the regolith-bedrock contact and the dye injection sites are close to the drainage divide between two groundwater basins. We had hoped for more rapid flow and we were encouraged by reports that Croft Spring at Grassmere responded like a karst-conduit, turbulent-flow spring, rising rapidly after storm events. However, our pressure-transducer, data-logger installed at Croft Spring shows that stage has been virtually flat with a rise of no more than 0.15 feet and virtually no fluctuation in temperature after rains since it was installed on 8-10-94. Of course, this is the driest time of the year and as indicated in the bid submitted for this project, dye traces should be performed during wet weather. Although, it has rained more than average during this period, that has not increased the discharge at Croft Spring. The stage at the CSX Outfall, the headwater of the East Fork of Browns Creek, has risen rapidly, as much as two feet after rains during this period. However, this is due to storm water runoff from Radnor Yard since this is the outfall for their storm sewer system. Groundwater also flows into this storm sewer system to discharge at the CSX Outfall. It is believed that the groundwater portion of the drainage probably has not fluctuated a great deal. This assumption is based on the recorded groundwater discharge at Croft Spring.

Perhaps groundwater discharge fluctuates more with rain during wet periods. The water table in the vicinity has been dropping during this period. This is indicated by the declining flow from Croft Spring and lower water levels in the monitoring wells and six piezometers at Radnor Yard. The water surface levels were several feet lower in these wells than they were in January, 1991, when they were measured by Resource Consultants in order to prepare the water table maps included as Figure 18 in their Phase I Report for Oil Pollution Abatement at Radnor Yard of CSX Transportation, Inc. (1991).

The following all tend to indicate that the reason none of the dyes have been detected at a spring is because of slow groundwater flow through the regolith portion of the aquifer:

1. Water table well above regolith-bedrock contact.
2. Injection near groundwater basin divide
3. Continuous stage and temperature data at Croft Spring indicate very little fluctuation during and after rains
4. Low groundwater levels and low aquifer recharge associated with driest time of year

5. Sinkholes and sinking streams, indicative of direct recharge into conduits in karst aquifers, are virtually absent
6. High Tinopal CBS-X concentrations remain in the Saad injection well and in the recent excavation on the Saad Site
7. The report prepared by Resource Consultants (1991, p. 65) indicates an average horizontal flow rate of approximately 132 feet per year based on rising head tests performed on four limestone piezometers using the Cooper, Bredehoeft and Papadopoulos method.

Therefore, we conclude that the dyes have not moved very far from the wells they were injected into. Perhaps as wet weather conditions arrive, probably in December, groundwater flow rates will increase and the dyes will be carried into conduits in the carbonate aquifer and then to a spring or springs.

EFFECTS OF ORGANIC COMPOUNDS PRESENT IN GROUNDWATER AT THE SAAD SITE ON DYES AND CHARCOAL DYE RECEPTORS

Because of some concerns about the possible effects of organic compounds (particularly hydrocarbons) in groundwater on dye used for the Saad Site and Radnor Yard traces, we have investigated to test this possibility. As stated at the Grassmere meeting on November 3, Crawford has performed over 800 dye traces since 1970, and most of these were at sites where there were large quantities of gasoline, diesel fuel, oil and many other contaminants. Many successful dye traces have been performed from sites that were more contaminated with hydrocarbons than the Saad Site to springs that were more contaminated with hydrocarbons than Croft Spring. In fact, he has never had a dye trace to fail because of organic compounds or other contaminants in the groundwater. The great majority of these dye traces were performed at toxic waste sites and routinely dyes were injected into groundwater where there was floating oil, gasoline and other non-aqueous phase liquids (NAPLs). Many traces have been performed where there were leaking underground storage tanks. Crawford has performed successful traces using Rhodamine WT and other dyes at sites where there were high concentrations of methanol and acetone at both the injection sites and at the receiving springs. When Crawford first started doing these kinds of traces, there were concerns that the organic contaminants might interfere with the dyes and prevent successful traces. Also, since chlorinated water breaks down the fluorescent dyes, there were concerns that the flush water might prevent a successful trace. Of course, there is minor interference with the dyes from the organic compounds and chlorinated flush water but the interference is inconsequential compared to the amount of dye that is sorbed onto clays and organics in the soil. Often during dye traces started from wells or pits excavated for dye injection in the soil, 80 percent or more of the dye is lost. Therefore, in order to overcome these losses, large quantities of dye are used. Much larger quantities than would be used during relatively easy traces started at sinking streams or sinkholes where the dye is injected directly into karst conduits.

It was obvious from well data that the water table in the Saad- Radnor Yard area was well above the top of bedrock and that the dyes would therefore have to travel slowly through the regolith for an unknown distance before entering a limestone conduit. Therefore, to overcome losses due to the dyes being sorbed onto clays in the regolith, very large quantities of dye were injected into both the well at Saad and the one at Radnor Yard. It is less than one-half mile to both the Croft Spring and the CSX Outfall (headwater of the East Fork of Browns Creek) from these wells. If these two injection sites had been sinking streams or open sinkholes, one pound of dye at each site would have been more than sufficient to perform these traces. One pound of Rhodamine WT would have turned Croft Spring and/or Browns Creek bright red. However, instead of one pound, 9 pounds of Rhodamine WT and 10 pounds of Tinopal CBS-X were injected into these wells on September 18, 1994. This certainly should have been more than enough to offset any minor interference from the oil and any other organics at these sites. Also, it was obvious from our background dye receptor and water sample analyses that oil and other organic compounds were not seriously effecting Fluorescein. The Fluorescein background levels were, and are, very high at CSX Outfall, Croft Spring, Radnor Yard monitoring wells, and at well B-11, the monitoring well used for dye injection at the Saad Site (the well with several inches of floating oil).

However, because of concerns expressed about organics, particularly hydrocarbons, possibly interfering with the dyes, an investigation was made (Appendix 1). The water used for the research was collected from the Saad Site from just below the floating oil in well B-11, the well used for dye injection. Rhodamine WT, Fluorescein, Tinopal CBS-X and Eosine were tested. The very high levels of organics do interfere with all four dyes to a limited degree, but on a much smaller scale than was believed to be the case. The three experiments indicate that the organics do not interfere appreciably with the dye's ability to fluoresce, the charcoal's dye acceptor site ability to bond with the dye anions, nor the charcoal's ability to release the dye during elution (Appendix 1). The very high concentrations of Tinopal CBS-X that remains in the Saad Site injection well (previous to additional dye injection) and in the oily water flowing into the excavated pit, indicate that the dye has not been severely affected by the organic compounds present at the site. The same is true for the Rhodamine WT injected into MW CSX-1 at Radnor Yard. These high levels also indicate that both dyes are moving very slowly through the regolith (fill). Also, the Eosine detected in the west monitoring well (MW SSS-1) at the Saad Site and then after 3 weeks, in the nearby Ogden Well at Radnor Yard indicates the slow movement of groundwater through the regolith. The Eosine levels on the charcoal have gradually decreased at MW SSS-1 on the Saad Site, and then after first being detected in the Ogden Well, they gradually increased and they are now gradually decreasing. Eosine has now been detected in a water sample collected from the excavated pit at the Saad Site. It was first detected on November 28, after over 2 inches of rain on November 27. Relatively high background fluorescein levels were detected in a water sample collected on November 22.

QUALITY ASSURANCE/QUALITY CONTROL

Because of the very low probability that organic compounds present in the groundwater at the sites and /or chlorinated water used to flush the dyes into the aquifer would prevent a successful dye trace, spikes are not included in our Quality Assurance/Quality Control (QA/QC) plan (Appendix 2). A check of recent QA/QC plans for three other leading consultants, involved primarily in dye tracing, indicates that they do not include spikes in their plans either. There are no EPA or ASTM standard methods for dye tracing and it should be expected that there are different opinions about procedures to be used. However, the procedures used by the major consultants are very similar and all should provide accurate dye traces. Crawford and Associates believe that they are using the best procedures and have an excellent QA/QC plan. Questions concerning the lab procedures and QA/QC should be addressed to Rick Tucker, Vice President and Lab Manager for Crawford and Associates, Inc. The lab typically runs about 300 to 400 dye receptors and/or water samples a week. Great care is taken both in the field and in the lab to insure that dye receptors and water samples are not inadvertently contaminated by dye. Although we do not believe that this has ever been a problem, it would be naive to assume that it could not happen. We believe the biggest problem in the dye trace procedures being used today is due to variation in background fluorescence on activated charcoal dye receptors. In performing hundreds of dye traces in industrial areas over the years, we have noticed large variations in backgrounds for all the dyes used. One of the advantages of using dye receptors is that dye is concentrated on the receptor so that even very small concentrations of dye in the stream collect on the receptor over time and thus permit detection. Unfortunately, fluctuating background levels are also concentrated on the receptor and this can result in a false positive. We believe that calling a dye trace positive based on one positive charcoal dye receptor could be a mistake since it could be due to background variation or possibly contamination in handling in the field or lab. Therefore, we require at least two positives and we prefer a quantitative trace where analysis of water samples, collected by an ISCO automatic sampler, permit one to actually quantify the amount of dye in the stream and to draw a dye breakthrough curve of the dye concentration as the dye cloud passes the sampler location. EPA has required that both a qualitative trace, using activated charcoal dye receptors, and a quantitative trace, using ISCO samplers, be used for this investigation. The dye breakthrough curve virtually eliminates the possibility of incorrectly calling a trace positive that might be due to variation in background, field or lab contamination or even sabotage. Requiring both a qualitative trace and quantitative trace represent the ultimate in QA/QC and assures that a trace will not be incorrectly called positive when it is not.

CHANGES IN SCOPE OF WORK

The original Scope of Work called for three dye traces to be run simultaneously:

1. Saad well (MW SSS-1)
2. A Radnor Yard well (MW CSX-1)
3. GE dye injection well

These wells were selected to provide information about groundwater basin divides. After one month, three additional traces were to have been started. The injection sites for these three traces were to have been selected based on results of the first three traces. Since none of the three dyes had been detected at any spring after six weeks, it was decided to wait before starting any of the proposed remaining three traces.

This increased flow through the regolith would result in more dye being sorbed onto clays as it passes through the regolith. The decision to use a small amount of flush water was made by Crawford due to the concerns expressed by other consultants that it might change the direction of groundwater flow. This decision was made to avoid controversy about the results of the dye traces. The purpose of the flush was to push the dye into a bedrock crevice faster and thus decrease the time required for the trace. The slow injection of even several thousand gallons of flush water over a period of days probably would not have increased the water table elevation more than a few inches 50 feet from the injection well and the chances of causing groundwater to flow into a groundwater basin that it does not normally flow to, at least on some occasions, were virtually zero. However, it was felt that the flush was not essential and that once the dye was injected into the groundwater flow, it would eventually reach a spring or springs. Therefore, only 300 gallons of flush water, instead of the usual several thousand gallons, were used at each site during the initial dye injection. However, in order to make sure that we had used enough dye to overcome the losses, it was decided to inject additional quantities of the same dyes and to increase the amount of flush water. It appeared obvious that after six weeks the dye had not been detected at a spring because of slow groundwater flow through the regolith.

DYE INJECTION

Saad Site At 2:30 p.m. on November 5, 1994, 10 pounds of Tinopal CBS-X (Optical Brightening Agent 351) were injected into MW SSS-1 at the Saad Site (Figure 1). This is the well located about 20 feet west of the shallow well initially used for dye injection, B-11 (Figure 2). This is the bedrock well that was originally selected for dye injection at the Saad Site. We changed to the shallow regolith well because the bedrock well had a capacity to take flush water of less than one gallon per minute. After injection of the 10 pounds of Tinopal, mixed with about 20 gallons of water, this well would only take about

2 1762

4" Stainless Steel Casing

8" PVC Casing

Cement Grout

HNu Background
Level: 1.2-2ppm

583.8

0

cap

Block

Land Surface

2

2 HNu=200ppm

4.5

5 HNu=50ppm LMS. BOULDER

10

7 HNu=120ppm

13.3

10 LDW 100%

570.5

WL after

16.6

16.6 HNu=50ppm

567.2

20

20 HNu=1ppm

30

Bigby-Cannon
Limestone

40

41.4 LDW 100%

539.8

44

538.2

45.6

Hermitage
Formation

Elevation
Above Mean
Sea Level

Feet Below
Land Surface

NOTE: Hydrogen Sulfide Smell
During Development with air.
Brown to Black water; foamy;
HNu=18ppm

Date Began: 9-14-82

Date Completed: 9-17-82

Vertical Scale: 1" = 10'

Horizontal Not To Scale

WLMP = TC

TC = 2.2FT > LLS



ecology and environment, inc.
4319 COVINGTON HIGHWAY, DECATUR, GEORGIA

SAAD SITE

NASHVILLE, TN.

CONSTRUCTION OF WELL 9881

TDD F4-8204-06

FIGURE A.7

FIGURE 1

PROJECT Saad Waste Oil
PROJECT NO. 37069190499 BORING B-11
ELEVATION 582.2 DATE 3/22/87
FIELD GEOLOGIST Karmazinski

[illegible]

REMARKS	Drilling Method - Auger
	Casing - Well Installed

BORING **B-11**

PAGE 1 OF 1

FIGURE 2

one-fourth gallon per minute of flush water. Although the flush water was set for this slow rate, it was turned off by Mr. Saad one or two times during the flush period. Therefore, virtually no water was used to flush this dye from the well into the aquifer. We believe this well was damaged during excavation of a nearby trench during previous investigations. Originally, it was 46 feet deep and had a capacity reported to be 50 gallons per minute (Figure 1).

GE Site After a preflush of 937 gallons of water, 3 pounds of Fluorescein (Color Index: Acid Yellow 73) were injected into the dye injection well at the GE Site at 2:43 p.m. on November 5, 1994. The well was then flushed at a rate of approximately 7.5 gallons per minute until 9:05 a.m. on November 6, 1994, for a flush of 7,176 gallons. The water table at the GE site is at least 30 feet lower than the groundwater drainage divide between Croft Spring and the CSX Outfall. Therefore, there were no concerns about causing dye to flow into a different groundwater basin (Plate 1).

Radnor Yard Site At 4:30 p.m. on November 5, 1994, 18 pounds (2 gallons) of Rhodamine WT (Color Index: Acid Red 388) were injected into MW CSX-1 at Radnor Yard. Because of problems with the faucet, the flush was not started until the next morning. It was then flushed with a total of 1,935 gallons of water at a rate of 1.25 gallons per minute from 10:11 a.m. on November 6, 1994, until 7:30 a.m. on November 7, 1994. Water surface elevations measured in other monitoring wells during the flush by Resource Consultants did not indicate any significant rises. Small rises in some wells, not necessarily the ones closest to MW CSX-1, were probably due to the 0.60 inches of rain that fell on November 5, 1994.

DYE ANALYSIS

Water samples collected by the ISCO samplers at Croft Spring at Grassmere and the CSX Outfall have been collected weekly and dye receptors have been exchanged every two weeks since November 5. The results do indicate the presence of Fluorescein at a spring east of the GE Site and low levels of Rhodamine WT in the storm sewer and CSX Outfall. However, we are waiting to look at additional dye receptors before calling either trace positive. About 2.03 inches of rain fell on November 27, 1994 and this may have a substantial impact on groundwater flow. The dry weather conditions which have existed since the beginning of this investigation may now be over.

APPENDIX 1
EFFECTS OF ORGANIC COMPOUNDS PRESENT IN
GROUNDWATER ON DYE USED AT SAAD SITE,
NASHVILLE, TENNESSEE

EFFECTS OF ORGANIC COMPOUNDS PRESENT IN GROUNDWATER ON DYE USED AT SAAD SITE, NASHVILLE, TENNESSEE.

INTRODUCTION

Due to the large amount of organics, particularly hydrocarbons, in the groundwater in the vicinity of the Saad Site, an investigation into possible interference with the dyes used in the dye trace was started.

EXPERIMENTAL PROCEDURE

Part One: Standards

To assess the extent of the interference, two sets of the following standards were made:

<u>DYE TYPE</u>	<u>CONCENTRATION</u>
Rhodamine WT	100 ppb
Fluorescein	50 ppb
Tinopal CBS-X	50 ppb
Eosine	100 ppb

One set of standards was made with de-ionized distilled water, the second with organic rich water collected at the Saad Site. After a thorough mixing, a sample of each standard was analyzed quantitatively on a Shimadzu Spectrofluorophotometer RF5000U. The following values were obtained:

<u>DYE TYPE</u>	<u>DI-WATER</u>	<u>ORGANIC RICH WATER</u>	<u>DIFFERENCE</u>
Rhodamine WT	100 ppb	92.5 ppb	-7.5%
Fluorescein	50 ppb	47.1 ppb*	-5.8%
Eosine	100 ppb	97.6 ppb	-2.4%
Tinopal CBS-X	50 ppb	45.3 ppb*	-9.4%

*Concentration after background fluorescence was subtracted.

This experiment was repeated after 2 hours to insure ample time for the organics to react with the dyes. The concentrations did not vary considerably from the above values.

Part Two: Bonding to Activated Charcoal

The second part of this experiment dealt with the organics ability to interfere with the bonding of the dye anions with the activated charcoal dye acceptor sites. To this end, one gram of activated charcoal was placed in 10 ml of each dye standard. After a period of 30 minutes, the liquid part of the sample was drained off and analyzed for fluorescence.

<u>DYE TYPE</u>	<u>DI-WATER SAMPLE</u>	<u>ORGANIC-RICH SAMPLE</u>
Rhodamine WT	0.001 ppb	0.002 ppb
Fluorescein	0.031 ppb	0.037 ppb
Eosine	0.004 ppb	0.007 ppb
Tinopal CBS-X	0.053 ppb	0.062 ppb

Part Three: Elution of Dye from Activated Charcoal

The third part of this experiment dealt with the possibility that the organics in the groundwater would interfere with the eluent's ability to remove dye from the charcoal. To assess the extent of this interference, the charcoal from part two of this experiment was eluted with 10 ml of eluent for a period of 30 minutes and the elutant then drained off and analyzed for each dye. The following values for peak wavelength/fluorescence intensity were obtained:

<u>DYE TYPE</u>	<u>DI-WATER SAMPLE</u>	<u>ORGANIC-RICH SAMPLE</u>
Rhodamine WT	567.6/761.3	568.2/720.4
Fluorescein*	516.8/48.1	516.6/132.7**
Eosine	536.8/605.9	536.8/596.8
Tinopal CBS-X	428.4/306.7	422.6/633.5***

Notes: *Low sensitivity scan was used.

**Background fluorescein could not be subtracted for this experiment.

***Background CBS-X could not be subtracted from this experiment.

RESULTS

The above data shows that the organics in the groundwater at the Saad Site do not interfere appreciably with the dye's ability to fluoresce, the charcoal's dye acceptor sites ability to bond with the dye anions, nor the charcoal's ability to release the dye during elution.

The data obtained from these three experiments show that the organics do interfere with the dye to a limited degree, but on a much smaller scale than was believed to be the case. The concentration of the CBS-X in the water collected from the site indicates that a large quantity of the dye remains completely unaffected by the organics in the groundwater at the Saad Site. As the groundwater travels from the Saad Site, the organics should become less of a problem for the dye as their concentrations will decrease. As long as a large quantity of dye is used for the trace, the organics will be unable to interfere to a measurable degree.

APPENDIX 2
CRAWFORD AND ASSOCIATES, INC.
LABORATORY PROCEDURES
AND
QUALITY ASSURANCE/QUALITY CONTROL PLAN

CRAWFORD AND ASSOCIATES, INC.

480 Peachtree Lane
Bowling Green, KY 42103

**LABORATORY PROCEDURES
AND
QUALITY ASSURANCE/QUALITY CONTROL PLAN****Sample Custody**

Water samples and charcoal and cotton dye receptors are received via overnight courier. Chain-of-custody forms are signed and the sample site name, date and time are recorded in a bound laboratory log book. One copy of the chain-of-custody form is added to the laboratory custody records and a second copy is taped to the sample container and it is locked in a refrigerator.

Sample Storage

Samples are kept in a locked refrigerator to which no one but laboratory personnel have access.

Documentation

Each dye receptor is kept in its original, labeled, sealed, polyethylene, zip-lock bag until it is removed from the refrigerator. The bags are opened one at a time and the receptor removed. It is washed in a high-speed jet of tap water to remove mud, a typed laboratory identification number and the original site location number are stapled to it and it is then placed in a dryer. A tag with a laboratory identification number and the original site location number is attached to each water sample bottle.

Instrumentation

After the elution of the activated charcoal receptors, the elutant is analyzed for dye on a Shimadzu Model RF5000U scanning spectrofluorophotometer. Water samples are also analyzed on the Shimadzu Model RF5000U. The unbleached cotton dye receptors are analyzed qualitatively for optical brightener and Direct Yellow 96 under a long-wave ultraviolet light.

Charcoal Testing

Preparation

- 1) Charcoal dye receptors are washed under a high-speed jet of tap water to remove as much mud as possible.
- 2) A typed laboratory identification number and the site location number are stapled to each receptor.
- 3) The receptors are placed in a fan dryer and air-dried for 24 hours.
- 4) One gram of charcoal is weighed and placed into a disposable plastic container which is labeled with the laboratory identification number and site location number.
- 5) The remainder of the charcoal is returned to its original zip-lock bag and, along with the cotton receptor, stored until the dye trace investigation is complete.
- 6) 10 ml of Smart solution (an eluent consisting of 1-propanol, de-ionized distilled water, and ammonium hydroxide mixed at a 5:3:2 ratio) is added to the charcoal and the plastic container is capped.
- 7) After 60 minutes, a 3.0 ml sample of the elutant is withdrawn from the plastic container using a disposable polyethylene pipette and placed into a disposable rectangular polystyrene cuvette.
- 8) The cuvette, with the elutant equilibrated to the ambient laboratory temperature, is placed in the Shimadzu RF5000U spectrofluorophotometer for analysis by synchronous scanning.

Analysis

There are no EPA or ASTM standard methods for the analysis of fluorescent dyes. However, Crawford and the three or four other scientists with the most experience in performing dye traces in karst aquifers are in agreement that analysis on a scanning spectrofluorophotometer provides the lowest detection limits and most reliable dye analysis. For a typical analysis for Fluorescein, Rhodamine WT, Eosine and/or Sulphorhodamine B in elutant by synchronous scanning, a Stoke's shift of 13 nm is used. The excitation scan is from 437 to 600 nm and the emission scan if from 450 to 613 nm. The scan speed is usually set on fast and the sensitivity is usually set on high. The excitation bandwidth for synchronous scans is

usually set at 3.0 nm and the emission bandwidth is usually set at 5.0 nm. The emission fluorescence from the synchronous scan is displayed on the monitor and plotted on pressure-sensitive chart paper. The laboratory identification number and site location number are immediately recorded on the original chart during analysis and the chart is then photocopied. The plot is modified by a 17-point smoothing routine on the Shimadzu. Another routine identifies the wavelength of the peaks to the nearest 0.1 nm and the magnitude (intensity) to the nearest 0.001 units.

Water Sample Testing

Preparation

- 1) Water samples are removed from locked refrigerator.
- 2) A typed laboratory identification number and the site location number are taped to each sample bottle.
- 3) Water samples are covered to prevent photochemical decay and permitted to equilibrate to the ambient laboratory temperature.
- 4) A 3.0 ml sample is withdrawn from the bottle using a disposable polyethylene pipette and placed into a disposable rectangular polystyrene cuvette.
- 5) The cuvette is placed in the Shimadzu RF5000U spectrofluorophotometer for analysis by synchronous or three-point scanning.

Analysis

The analysis for water samples is performed by synchronous or three wavelength scanning on the spectrofluorophotometer. The synchronous scanning technique is similar to the analysis for eluted charcoal samples with the scanning parameters adjusted to compensate for shifts in the excitation and emission maximum wavelengths as well as differences in the Stoke's shift caused by the differences in pH and polarity of water as compared to elutant. The three wavelength scanning technique uses three different excitation/emission wavelength pairs with a common Stoke's shift to measure fluorescence intensity, subtract background fluorescence, and calculate concentration from a calibration curve of known concentrations. Since dye concentrations in water samples truly represent the actual concentration of dye in the water at the time of sampling, different parameters are used to scan for each dye as to ensure accurate quantitation. The excitation bandwidth is set to 3.0 nm

and the emission bandwidth is set to 5.0 nm for both techniques. When using the three wavelength scanning method, 10% of the samples are also scanned synchronously to verify that the fluorescence is actually coming from the dye being analyzed and not from background sources. Samples with the highest and lowest concentrations are included in this 10%. All synchronous scans and the data sheets generated by the three wavelength method are labeled with the appropriate laboratory identification number and site location number during analysis and photocopied.

Cotton Testing

Cotton dye receptors are washed under a high-speed jet of tap water to remove as much mud as possible and then observed under a long-wave ultraviolet light and visually compared to cotton standards for optical brightener and Direct Yellow 96. The cotton receptors are removed from the original, labeled, zip-lock bag one at a time, analyzed under the ultraviolet light, and then immediately returned to the zip-lock bag. Optical brightener and Direct Yellow 96 are often analyzed for on activated charcoal elutant samples and in water samples using the spectrofluorophotometer with the appropriate parameters for each dye.

Dye Quantification

Standards

Standards are prepared from the most recent dye batch supplied to Crawford and Associates by the suppliers. The standards for analysis of water samples are prepared in bicarbonate water made in the laboratory from distilled, de-ionized water that has been adjusted to closely resemble water from the study area. Standards for eluted charcoal sample analysis are prepared in the eluent to be used for eluting the dye from the charcoal. This is usually the Smart solution since research by Crawford and Associates (Turner, 1993) indicates that it elutes more dye from the charcoal than other eluents tested. The dye concentration in the dye sample used for standard preparation is based upon the activity figure provided by the dye manufacturer. Rhodamine WT has an activity of 20% therefore its actual measurable concentration is 20% of its calculated concentration. Fluorescein usually has an activity of around 78%, eosine's activity is usually 80%, and optical brightener usually has an activity of 97%, but each varies with lot and manufacturer.

Measurement of Dye Concentration

The dye concentrations in eluted charcoal samples are measured from plots of emission wavelengths produced by the synchronous scans. The actual dye concentrations are calculated by using the following procedure:

- 1) The three point method is used to separate dye fluorescence from background fluorescence.
- 2) The dye fluorescence intensity is calculated by subtracting background fluorescence intensity from total fluorescence intensity at the appropriate emission wavelength.
- 3) The dye concentration is obtained from a calibration curve created by plotting fluorescence versus concentration from a set of known standards.

The dye concentration in water samples is determined by the three wavelength method which tends to remove the influence of background fluorescence. Dye concentrations are expressed in ppb. Although the dye concentration in water samples expressed in ppb is an accurate quantitative measurement of the amount of dye in the stream at the time the sample was collected, the same is not true for the eluted charcoal samples. It is only semi-quantitative compared with the actual quantity of dye in the water passing over the receptor. The quantity of dye absorbed by the charcoal is a function of the dye concentration in the water and the quantity, velocity, temperature and duration of exposure. Turbidity and the quantity and species of molecules competing with the dye for the charcoal acceptor sites can reduce the quantity of dye absorbed onto the charcoal. Also, the quantity of dye eluted from the charcoal is dependent on the amount of charcoal and eluent used, the type of eluent, whether the charcoal is wet or dry before elution, and the length of time the charcoal is eluted before being analyzed. The laboratory procedures can be standardized but the exposure variabilities while the receptor was in the stream cannot be. Although, dye concentrations for eluted samples are measured and recorded in ppb (or often just intensity), these values will virtually always be much higher than the dye concentrations ever reached in the stream. Also, because of the several water exposure variables, the concentration of dye absorbed by the charcoal does not even accurately represent the quantity of dye that flowed in the stream past the dye receptor. Analysis of two dye receptors placed in the same general area of the same stream for the same time period will often result in large differences when expressed in ppb. Therefore, the following abbreviations are used to express the dye concentration in more general

terms rather than ppb:

- Negative
- + Positive
- ++ Very Positive
- +++ Extremely Positive
- B- Weak Background
- B Background
- B+ Strong Background
- 0 Dye receptor not recovered

Although dye concentration cannot be accurately quantified from charcoal dye receptors, detection of dye at any concentration above background fluorescence does constitute a positive trace. If a quantitative dye trace is necessary, it must be based on dye analysis of water samples, not charcoal. Crawford and Associates frequently perform quantitative traces by collecting water samples with an ISCO automatic water sampler. This method provides a dye breakthrough curve which is an accurate measurement of the dye concentration in the stream as the dye cloud passes the monitoring site.

Quality Control Samples

Field Duplicates

Duplicate dye receptors are placed at 20 percent or more of the sites to be monitored. The second receptor serves as a back-up in the event that the primary receptor is lost or stolen. The secondary receptors are analyzed as duplicates for at least 10 percent of the total dye receptor locations.

Trip Blanks

At least two trip blanks are prepared in the laboratory and sent along with the new dye receptors to the field. The trip blanks are then kept with the old dye receptors as they are being collected in the field, returned to the laboratory, and analyzed.

Laboratory Blanks

Eluent Blank- Each batch of eluent solution is analyzed for each dye before it is used to elute charcoal samples.

Charcoal Blank- A sample from each new one-pound can of activated charcoal is eluted and analyzed for each dye before the remainder of the charcoal in the can is used.

De-ionized Distilled Water Blank- A de-ionized distilled water sample is analyzed to demonstrate that the dye signal on the chart produced by the spectrofluorophotometer is not generated by the instrument. One such blank is analyzed before and after each set of samples. One is also analyzed after every 20 samples.

Laboratory Control Standards

A low concentration standard, at the detection limit for each dye to be analyzed is analyzed before and after each set of samples. This demonstrates that the Shimadzu is capable of detection at the minimum detection limit. One is also analyzed after every 20 samples.

Analysis Order

Analysis will be performed in the general sequence of no dye to low dye to high dye concentration as follows:

1. De-ionized distilled water blank
2. Eluant blank
3. Charcoal blank
4. Laboratory control standard for each dye
5. Trip blank
6. Samples of presumed low concentration
7. Samples of presumed high concentration
8. De-ionized distilled water blank
9. Laboratory control standard for each dye